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Impact of age, gender and race on circulating gammadelta T cells

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Abstract

A major subset of human peripheral blood $\gamma\delta$ T cells expresses the V γ 2V δ 2 T cell receptor (TCR) and responds to malignant or infectious diseases. We noted significant differences in the numbers of V γ 2V δ 2 T cells in blood samples from healthy Caucasian or African American (AA) donors. On average, CA donors had $3.71 \pm 4.37\%$ V δ 2 cells (as a percentage of total lymphocytes) compared to $1.18 \pm 2.14\%$ V δ 2 cells for AA donors ($p < 0.0001$). Age and race had the greatest impact on V δ 2 cell levels; the effect of age was similar for both racial groups. The V δ 2+ cell population was dominated, for both donor groups, by cells expressing the V γ 2-J γ 1.2 V δ 2 T cell receptor, an apparent result of strong positive selection and there was substantial overlap in the public V γ 2 clonotypes from both racial groups. Mechanisms for selection and amplification of V δ 2 cells are nearly identical for both groups, despite the significant difference in baseline levels. These data show that appropriate controls, matched for age and race, may be required for clinical studies of V γ 2V δ 2 T cells in infectious disease or cancer and raise important questions about the mechanisms regulating the levels of circulating V δ 2 cells.

INTRODUCTION

Within the U.S. population, circulating lymphocyte levels [1] and the proportions of CD4+ or CD8+ T cells [2] are nearly identical among Caucasian (CA) and African American (AA) individuals. Racial groups vary in their levels of circulating neutrophils, but these differences are modest reviewed in [1,3]. On the other hand, differing baseline neutrophil counts may impact dosing decisions for breast cancer chemotherapy, especially among AA women [4]. It is important to understand race-dependent variation in leukocyte populations, and to determine whether matched control groups are required for clinical studies. Here, we investigate the effect of age, gender and race on the circulating levels of V γ 2V δ 2 T cells.

The T cell subset expressing the V γ 2V δ 2 T cell receptor (also designated V δ 2 cells) is distinct from the more familiar CD4+ or CD8+ T cells that express an $\alpha\beta$ T cell receptor. Humans have three V δ segments that generate the adult repertoire. V δ 1 chains pair with most of the V γ chains and dominate the intraepithelial subset of mucosal $\gamma\delta$ T cells where the TCR recognizes stress

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molecules on epithelial cells [5,6]. V δ 3 cells are less well studied, are found in blood at low levels and may be associated with protective responses against herpesviruses [7]. The V δ 2 chain is mainly paired with V γ 2 in blood lymphocytes from adults, comprising 60–90% of circulating $\gamma\delta$ T cells [8–10]. The majority of V γ 2 chains contain the J γ 1.2 segment [11,12]; this rearrangement encodes recognition of phosphoantigens [13] that are intermediates in mammalian or prokaryotic synthesis of isoprenoids [14–16]; phosphoantigen recognition is MHC and CD1-independent [17]. It has been proposed that the chronic presence of phosphoantigens drives extrathymic selection and amplification of a V γ 2-J γ 1.2 V δ 2 subset [18] and it was reasonable to assume that these same mechanisms controlled the normal blood levels of V δ 2 cells.

Without MHC restriction, the phosphoantigen-reactive V γ 2V δ 2 repertoire is similar among most individuals. There is a strong bias toward the V γ 2-J γ 1.2 rearrangement and a high frequency of public V γ 2 chains. It is even possible to find public V γ 2 chain nucleotypes (identical CDR3 region nucleotide sequences) [19]. Thus, positive selection by phosphoantigen and the absence of MHC restriction creates a V γ 2 chain repertoire that is dominated by public clonotypes. These findings also support the view that selection and amplification by ubiquitous phosphoantigens control repertoire composition and V γ 2V δ 2 T cell levels in healthy adults. There are several examples that demonstrate the relationship between phosphoantigen levels and V δ 2 cells. Exposure to extraordinarily high levels of phosphoantigens, that occurs in *Plasmodium* [20], *Brucella* [21], *Francisella* [22,23] or *Mycobacterium* [24–26] infections, increase V γ 2V δ 2 T cells to more than 30% of total lymphocytes. In non-human primates, synthetic phosphoantigens elevated blood V γ 2V δ 2 cell counts [27] and exposure to mycobacterium BCG increased cell levels and altered the V γ 2 chain repertoire [28]. However, V δ 2 counts generally return to basal levels after the exposure to phosphoantigens.

Caccamo and colleagues reported gender and age effects on blood V δ 2 levels, with women having lower levels compared to age-matched men [29]. Hviid's group reported inversion of the V δ 2:V δ 1 cell ratio in healthy volunteers in Ghana who were negative for malaria and HIV disease [30]. In a control group for our HIV studies in China, we observed V δ 2 cell levels nearly identical to Caucasian populations in the United States [31]. These studies begin to show that V δ 2 cell levels may be subject to multiple control mechanisms and there may be population differences.

Now, we have examined the levels, phenotypes and functional responses of V δ 2 cells in healthy, adult African Americans (AA) living in and around the city of Baltimore, MD, and compared them to age and gender-matched healthy Caucasian (CA) donors. We uncovered substantial differences between AA and CA donors. To test the hypothesis that V δ 2 populations are controlled by responses to phosphoantigens, we characterized the spectrum of public V γ 2 chain clonotypes in both donor pools and performed *in vitro* antigen stimulations. These data provide a comprehensive view of V δ 2 cell differences between AA and CA donors, and suggest that simple positive selection models will not account for the overall control of V δ 2 cells in humans.

MATERIALS AND METHODS

PBMC Isolation and V γ 2V δ 2 Stimulation

Heparinized venous blood was collected from 33 self-reported Caucasians and 32 self-reported African American HIV-negative and healthy volunteers. HIV status was self-reported and confirmatory tests were not administered. The study was approved by the Institutional Review Board at the University of Maryland, Baltimore, and written informed consent was obtained from each donor. Peripheral blood mononuclear cells were purified and cultured as described previously [32].

Stimulation index (SI) represents the proportional increase in V δ 2 cells following IPP stimulation compared to control with IL2 and no IPP. SI is a ratio of the absolute number of V δ 2 lymphocytes on day 14 of the IPP expansion to the absolute number of V δ 2 lymphocytes on day 14 of the IL2 alone expansion.

Flow cytometry

Ex vivo PBMC or expanded V γ 2V δ 2 T lymphocytes were resuspended in PBS (Gibco)-10% FBS (Gibco) and stained at 4°C with directly conjugated monoclonal antibodies for the detection of cell surface markers. Granzyme B was detected by intracellular staining using the Cytofix/Cytoperm Kit from BD Biosciences according to manufacturer's instructions.

Flow cytometry data were collected on a FACSCalibur (BD Biosciences, San Diego CA). At least 3×10^4 lymphocytes (gated on the basis of forward and side scatter profiles) were collected for each sample and results were analyzed with FlowJo software (Tree Star, San Carlos CA). The following monoclonal antibodies were used for four-color staining: anti-V δ 2 FITC, clone B6 (BD); anti-CD95 PE, clone DX2 (BD); anti-CD3 APC, clone UCHT1 (BD); anti CD45Ra PC5, clone HI100 (BD); anti-CD16 PC5, clone 3G8 (BD); anti-CD3 PerCP, clone SP34-2 (BD); anti CD56 PE and anti CD56 APC, clone N901 (Beckman Coulter); anti-Granzyme B, clone GB12 (Caltag, Burlingame CA); anti-CD27, clone O323 (eBioscience, San Diego CA); and the appropriate isotype controls (all purchased from BD Biosciences).

RNA extraction, RT-PCR, PCR

RNA extraction and cDNA synthesis were performed as described [33] using the following primers for the V γ 2 (V γ 9 according to the IMGT nomenclature) chain: oligo V γ 2 (5' ATC AAC GCT GGC AGT CC 3') and oligo C γ -1 (5' GTT GCT CTT CTT TTC TTG CC 3').

Run-off reaction

Primer extension reactions and analysis of the length distribution of the V γ 2 chain CDR3 region were performed as described [32]. Run-off product lengths were corrected by adding the length of the known mRNA coding region outside the run-off primer-binding site. According to our convention [32] the V γ 2 chain population has a peak length at 993 nucleotides.

Repertoire analysis

We evaluated the V γ 2 chain repertoire among representative AA donors. mRNA were prepared from PBMC and individual V γ 2 chain sequences were cloned into plasmids as described [34]. DNA sequences surrounding and including the CDR3 regions were determined for 150–300 plasmid clones from each donor. Sequences were aligned, we determined how many sequences were repeated in the library from each donor, and we generated amino acid sequences for comparison. Data were expressed as the proportion of V γ 2-J γ 1.2 rearranged sequences among all V γ 2 chains, with average values calculated for 8 AA donors and 6 CA donors. We also identified public clonotypes within the AA group and deduced the frequency of appearance for individual public clonotypes in each donor. Data for CA donors were derived from our existing database of nearly 50,000 human V γ 2 chain sequences. The CA and AA donors were matched for age and gender.

Statistical analysis

The student t test was used to compare the mean difference when a Gaussian distribution was present; otherwise (e.g., when there were outliers), the Wilcoxon rank sum test was used to compare two groups. The multi-variable analysis was performed with the mixed procedure using SAS 9.2 software (SAS Institute Inc., Cary, NC). Non-normal data were normalized (e.g., log-transformed) before analysis. $P < 0.05$ was considered to be significant.

RESULTS

Characteristics of the donor population

We obtained blood specimens from 32 African American (AA) donors and 33 Caucasian (CA) donors. The mean age was 43.6 years (19 – 63 years) and 41.8 years (26 – 64 years) for AA and CA donors, respectively. The AA group included 19 females and 13 males; the CA group included 19 females and 14 males. Data for all donors are presented in Tables 1 and 2.

Baseline values for V γ 2V δ 2

Baseline values were measured in PBMC specimens. We evaluated by flow cytometry, the percentage of V δ 2 T cells among total lymphocytes. Total lymphocyte counts are very similar among AA and CA donors in the U.S. [1,35,36]. Thus, percentage should be valid for our comparisons. The range for all donors was 0.03% to 21.3% V δ 2 cells in total lymphocytes; with ranges of 0.16–21.3% for CA donors and 0.03–11.4% for AA donors (Fig. 1). Mean and median, for the percentage of V δ 2 cells in total lymphocytes were 3.71% and 2.25% for CA donors, with a standard deviation of 4.37% (Fig.1); and 1.18% and 0.43% for AA donors, with a standard deviation of 2.14% (Fig.1). The mean and median were 3.1 fold and 5.6 fold higher, respectively in CA compared to AA. Three outliers were identified in the boxplot analysis. The Wilcoxon test revealed a significant difference in the distribution of V δ 2 numbers between CA and AA ($P < 0.0001$). If outliers were excluded, the difference was still significant ($P < 0.0001$).

Multivariate analysis by mixed effect models

We next sought to determine whether the racial difference in V δ 2 cell frequency is significant after adjusting for age and gender. Race ($P < 0.0001$) was still a significant predictor of log (V δ 2) values after adjusting for age and gender. Age itself was also associated with log(V δ 2 +) ($P = 0.022$), whereas gender was not ($P = 0.34$). There were no interactions between race and age ($P = 0.28$). The percentage of V δ 2+ cells declined as age increased, and rates of decline were the same for CA and AA donors.

We extended the multivariate analysis to examine whether phenotype markers or the V γ 2 chain repertoire depended on race, age and gender. CD56 is a marker identifying the sub-population of V γ 2V δ 2 T cells that is most potent in cytotoxicity assays [19,37]. Neither gender ($P = 0.41$) nor age ($P = 0.24$) affected the proportion of CD56+ V δ 2 cells. However, race was a significant predictor of CD56 cell levels ($P = 0.0031$). There were no significant interactions between race and gender ($P = 0.64$). CD56 was expressed on $45.3\% \pm 16.1$ of V δ 2 cells from CA donors (Fig. 2) compared to $29.8\% \pm 18.3$ from AA donors ($P = 0.0013$).

Next, we evaluated the proportional distribution of naïve (CD27+/CD45RA+), T central memory (CD27+/CD45RA-), T effector memory (CD27-/CD45RA-) and T effector memory RA (CD27-/CD45RA+) subsets among V δ 2+ T cells [38,39]. For all of these values, there were no significant interactions between race and gender. Neither race, age nor gender were significant predictors of T effector memory cells. Only race was a significant predictor of the TemRA compartment ($P = 0.033$). Age was a significant predictor for the proportion of Naïve cells ($P = 0.049$). Race ($P = 0.012$), gender ($P = 0.030$) and age ($P = 0.019$) were significant predictors of the T central memory compartment (Table 3).

The value of V γ 2₉₉₀₋₉₉₆ represents the proportion of V γ 2 chains in a single specimen, with mRNA coding region lengths between 990–996 nucleotides. This value closely approximates the proportion of V γ 2 chains containing the V γ 2-J γ 1.2 rearrangement [32] which is associated with responses to phosphoantigen stimulation [13]. The V γ 2₉₉₀₋₉₉₆ was 0.70 ± 0.22 for CA donors with values ranging from 0.06 to 0.94 (Fig. 3). The V γ 2₉₉₀₋₉₉₆ was 0.57 ± 0.25 for AA donors with values ranging from 0.06 to 0.9 (Fig. 3). Neither race, age nor gender were

significant predictors for the $V\gamma_{2990-996}$. However, if only race was included in the model, it became a significant predictor ($P = 0.046$).

Other markers of V δ 2+ T cells

Granzyme B (GrB) is another marker for cytotoxic T cells. We characterized Granzyme B production and tested V δ 2 lymphocytes for proliferation responses to phosphoantigen. These tests were only done on a subset of samples from the donor pool and were not included in the multivariate analysis. Among CA donors, $57.1\% \pm 16.9\%$ of V δ 2 cells expressed GrB, compared to $42.6\% \pm 20.4$ for AA donors ($P = 0.012$) (Fig. 4). The difference was in line with our finding from the multivariate analysis that only race was a significant predictor of the TemRA compartment where GrB expression is most frequent [40].

The proliferation response to phosphoantigen, represented by the stimulation index, is a measure of functionality for V δ 2 cells in human PBMC. Stimulation index is defined as: (absolute number of V δ 2 cells after phosphoantigen + IL2) $\div$ (absolute number of V δ 2+ cells after IL2). The mean stimulation index for CA donors was 33.5 ± 31.0 , with values ranging from 5.5 to 97 (data not shown). For AA donors, the mean stimulation index was 35.2 ± 46.4 with a range from 1.7 to 160.3 (data not shown). There was no significant difference in the mean values between the two groups. Our results indicate that V δ 2 cells from AA or CA donors have similar capacities for responding to phosphoantigen stimulation.

Phosphoantigen stimulation also increased the proportion of CD56+ V δ 2 T cells. After phosphoantigen stimulation, the CD56+ subset was $67.7\% \pm 13.9\%$ for CA and $50.2\% \pm 20.7\%$ for AA donors ($p < 0.05$, data not shown). The proportional increases in CD56+ V δ 2 cells after phosphoantigen stimulation were similar for AA (1.7 fold) and CA donors (1.5 fold), consistent with the stimulation index data.

Characteristics of the V γ 2 chain repertoire

We used molecular cloning to evaluate the V γ 2 chain repertoire in multiple donors. Plasmid libraries were constructed from 8 AA and 6 CA donors; sequences were obtained for the V-J regions. Sequence data identified J segments used in each chain and defined the non-templated nucleotides in each CDR3 region. Within the library of sequences for each donor, we identified sequences present more than once (repeated). Amino acid sequences (clonotypes) that were present in more than one donor (identical at all positions within the V, N and J regions) were defined as public clonotypes.

Public clonotypes represent the most commonly selected V γ 2 sequences. The profile of public clonotypes can reveal similarities or differences in the V γ 2 T cell repertoire that in turn, reflect the selection and amplification mechanisms.

For 8 AA donors, 29 public clonotypes were identified (Table 3); 16 sequences were common to 2 donors (55%), 5 were common to 3 donors (17%), 2 were common to 4 donors (7%), 3 were common to 5 donors (10%), 2 were found in 6 different donors and 1 clonotype was found in all 8 donors. The public clonotype found in all donors (CDR3 region sequence of CALWEVQELG, called the canonical sequence) represents the germline configuration of V γ 2 and J γ 1.2 segments without deletion/addition of non-templated nucleotides during DNA recombination. For this collection of public clonotypes, 21 of 29 (72%) were also found in the list of public clonotypes for CA donors (Table 4).

Among CA donors we identified 51 public clonotypes. 30 public clonotypes (59%) were found in 2 donors, 9 clonotypes were in 3 donors (18%), 8 were found in 4 donors (16%), 1 was found in 5 donors and 3 were found in all 6 donors. All of the clonotypes found in at least 5 of the CA donors (including the canonical sequence) were also present in AA donors; only 3 of the

common clonotypes (in at least 4 of 6 CA donors) were not on the list of public clonotypes for AA donors.

The pattern, abundance and distribution of public V γ 2 chain clonotypes were similar among AA and CA donor groups. The larger number of public sequences found in CA donors probably reflects the larger number of total V γ 2-J γ 1.2 sequences obtained for each sample. Generally, public clonotypes unique to either group were less abundant (fewer repetitions) and present in fewer donors within the group. The most common clonotypes were present in both groups. Overall, the V γ 2 chain repertoire in AA and CA donors suggests strongly that selection and amplification mechanisms are similar in these groups, resulting in substantial overlap in the public clonotypes.

DISCUSSION

Human $\gamma\delta$ T cells are derived from double negative thymic precursors, like $\alpha\beta$ T cells, but $\gamma\delta$ T cells develop along a separate pathway that does not involve MHC-restriction and does not generate lineages that are marked by expression of cell surface CD4 or CD8 glycoproteins. Most importantly, there is no known negative selection step during $\gamma\delta$ T cell ontogeny; cells capable of recognizing self-antigens are released to the periphery.

In the absence of MHC restriction and thymic negative-selection, extrathymic selection and amplification drives repertoire maturation [18]. One product of extrathymic selection is a repertoire dominated by cells expressing the V γ 2V δ 2 receptor [8–10] and mostly including the V γ 2-J γ 1.2 rearrangement [11,12]. The common presence of phosphoantigens and the strong responses of V γ 2-J γ 1.2 V δ 2 cells are believed to be the force behind extrathymic selection and amplification of the V γ 2V δ 2 T cell subset. However, the mechanisms controlling steady state levels of V γ 2V δ 2 T cells have not been explored. Unlike circulating $\alpha\beta$ T cells, the V δ 2 subset is mostly composed of antigen-experienced, positively selected memory cells with potent effector functions including chemokine or cytokine release [41–43] and cytotoxicity [44]. Naïve cells are rare in this population [45].

We used a multivariate model to test the impact of age, gender and race on V δ 2+ T cell levels. Age was negatively associated with V δ 2 T cell levels similarly to what was reported by Caccamo et al. [29]. In our study, age-related decline of V δ 2 cells were the same for CA and AA groups and there were no significant effects of gender. However, larger sample sizes might have revealed an impact of gender similar to that reported by Caccamo, et al. [29]. Race was the most important factor affecting V δ 2 cell levels.

The differences in V δ 2 cell numbers were consistent with a lower proportion of CD56+ cells, and lower granzyme B expression in the V δ 2 cell subset of AA donors. Differential granzyme expression is possibly related to a shift in the distribution of V δ 2 cells across different memory compartments. Granzyme B is expressed at higher levels in the terminally-differentiated TEMRA cell subset (CD27– CD45RA+) [38] which is lower (8.3% versus 13.26%) in AA donors. All of these differences might have been consistent with decreased strength of response to phosphoantigens due either to alterations in the signal transduction pathway, differences in antigen presentation or differences in TCR structure and repertoire. Within the limits of our data, we did not find substantial differences in the stimulation index for phosphoantigen in AA versus CA groups. Based on this finding, we felt it was unlikely that antigen presentation or signal transduction would differ among groups.

We also evaluated the complexity of V γ 2-J γ 1.2 chains, looking for the degree of clonality and the presence of public clonotypes that might reveal differences between our donor groups. In the V γ 2-J γ 1.2 sequences from 8 AA donors, we identified 29 clonotypes that were found in 2 or more donors. For CA donors, we identified 51 sequences present in 2 or more donors. The

larger number of public clonotypes identified in the CA group is likely due to a larger yield of molecular clones with the V γ 2-J γ 1.2 sequence obtained for each sample. There was a substantial overlap between AA and CA public repertoire, with 72% of public V γ 2 chains from the AA group being identical to public chains in the CA group. The reverse was also true, with 41% of CA public chains also identified as public clonotypes in the AA group. Importantly, the common public chains (defined by their presence in most or all donors) were very similar in both groups. We believe that overlap among public clonotypes provides the strongest evidence for similar selection and amplification of V γ 2V δ 2 cells in both donor groups. Accordingly, we do not find substantial differences in TCR structure or repertoire selection that can account for the population differences in baseline values or phenotypic variation.

The existing models for V γ 2V δ 2 T cell repertoire maturation focus on selection and amplification driven by common phosphoantigens. One might extrapolate from these models, that the same forces shaping the repertoire also control the circulating levels of V γ 2V δ 2 T cells. Our results show that selection/amplification do not control the circulating cell levels. The differences in CD56 or Granzyme B expression, and the change in proportion of TemRA cells all point to differences in T cell homeostasis among the groups studied here. These data do not imply a functional defect in V δ 2 cells for the AA group. The key is that proliferation responses to phosphoantigen, which were similar among the groups, would rapidly mitigate any baseline differences. Our data do show group-specific differences in the distributions of memory cell populations that might be related to the control of cell numbers.

Our studies do not address the myriad of potential environmental factors that might influence T cell homeostasis. All donors in this study claimed to be in good health and all lived in and around the metropolitan area of Baltimore, Maryland. Beyond this level, we have no information on their prior exposure to environmental agents that might impact V δ 2 T cell levels. We have limited data on populations other than individuals living in Baltimore or Southern China. We have some information for adult women in Cameroon ($n = 28$) who had median V γ 2V δ 2 T cell levels intermediate between the AA and CA groups reported here, but these women were not age-matched to the groups in our study (mean age: 28 ± 5 years) and were all at the end of pregnancy. Nonetheless, we recognize that our results might be influenced by using only a local population. Larger studies are needed to determine whether the impact of race on V γ 2V δ 2 T cell levels can be generalized, including studies of additional racially or ethnically distinct populations.

It is unusual to have persistent, memory T cell populations focused on a single antigen. The example of V γ 2V δ 2 T cells is similar to human invariant NKT cells that recognize CD1-restricted galactosylceramide molecules [46] and all express the identical V α 24 chain of the T cell receptor [47]. The $\gamma\delta$ T cell repertoire does include a substantial proportion of identical V γ 2 clonotypes but in contrast to iNKT cells, the population is clonally diverse and there is a broad range for the strength of response to phosphoantigen [48]. These features, clonal diversity but redundant phosphoantigen recognition, arise from the chronic, positive selection mechanism first proposed by Brenner's group [18]. However, this mechanism does not account for differences in baseline levels that we report here. Human V δ 2 cell populations have the interesting feature of very few naïve cells and a stable repertoire [49] dedicated mainly to phosphoantigen recognition. Thus, studies on V δ 2 cells may provide insight into the control of T cell memory that may be relevant to natural and acquired immunity to pathogens in distinct population groups.

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Figure 1.

Vδ2 cell levels are lower in AA donors than in CA donors. Vδ2 frequencies were compared for 33 Caucasian (CA) and 32 African American (AA) donors. The scatter-plots show individual values as well as mean $\pm$ standard deviation for each group of donors. The Vδ2 frequency was $3.71\% \pm 4.37\%$ (median 2.25%) for CA donors and $1.18\% \pm 2.14\%$ (median 0.43%) for AA donors. P value is the result of Wilcoxon test.

Figure 2.

The fraction of CD56+ Vδ2 cells is lower for AA donors than for CA donors. The frequencies of CD56+ Vδ2 cells were compared between Caucasian and African American donors (Mean $\pm$ SD). CD56 was expressed on 45.3% $\pm$ 16.1 of Vδ2+ cells from CA donors compared to 29.8% $\pm$ 18.3 from AA donors. The scatter-plots show individual values as well as mean $\pm$ standard deviation for each group of donors. P value is the result of an Unpaired t Test.

Figure 3 A

Figure 3 B

Figure 3.

The fraction of V γ 2-J γ 1.2+ is lower for AA donors. RNA was collected from PBMC from Caucasian (n = 25) or African American (n = 26) donors. Following V γ 2 PCR amplification and runoff analysis, relative frequencies were averaged for each chain length. (A) Histogram plots show mean and standard deviation for every V γ 2 chain length, for each group of donors. (B) The fraction of chains with a length between 990–996 nucleotides (J γ 1.2) was on average 0.57 ± 0.25 among AA donors and 0.70 ± 0.22 among CA donors. The scatter plot shows for both groups individual V γ 2 990–996 values as well as mean $\pm$ St Dev. P value is result of a Wilcoxon Test.

Figure 4.

Lower Granzyme B expression in Vδ2 cells from AA compared with CA donors. The frequencies of Granzyme B+ Vδ2 cells were $42.6\% \pm 20.4\%$ for AA donors and $57.1\% \pm 16.2\%$ for CA donors. The scatter-plots show individual values as well as mean $\pm$ standard deviation for each group of donors. P value is the result of an Unpaired t Test.

Table 1

Caucasian Donors

Sample ID	Sex	Age
ND001	M	54
ND003	M	27
ND004	M	57
ND005	M	37
ND006	F	32
ND007	F	28
ND008	M	30
ND010	F	32
ND014	M	34
ND015	F	26
ND016	F	57
ND017	M	32
ND018	F	27
ND019	F	29
ND020	F	29
ND038	F	64
ND039	M	44
ND040	M	43
ND041	M	40
ND042	M	41
ND043	M	47
NB23	F	43
RF32	F	43
AT49	F	57
AD58	F	48
LH61	M	52
CB62	F	54
MH66	F	41
KH68	F	39
ME71	F	50
SE72	M	53
DW74	F	50
MV50	F	41
Mean		41.88
St.Dev		11.28
Median		41.00

Table 2

African-American Donors

Sample ID	Sex	Age
ND023	F	24
ND024	F	45
ND025	F	26
ND026	F	58
ND027	F	41
ND028	M	50
ND029	M	57
ND030	M	62
ND031	M	39
NVS101	M	55
NVS102	F	44
NVS104	M	59
NVS105	F	49
NVS107	F	47
NVS109	F	48
NVS112	M	43
MP063	F	63
MP068	F	51
MP089	M	53
CH46	F	35
EG41	F	52
KP42	F	59
HR45	M	49
LR33	F	31
CF37	M	47
SF48	F	19
YL43	F	47
ND036	M	35
ND037	M	25
ND044	F	29
ND045	M	30
ND046	F	24
Mean		43.63
St.Dev		12.49
Median		47.00

Table 3

Distribution of Vd2 T cells among naïve/memory compartments

	Naïve CD27+/CD45RA+	CM CD27+/CD45RA-	EM CD27-/CD45RA-	EMRA CD27-/CD45RA+	
CA (N=32)	15.12	46.50	25.11	13.26	Mean
	17.64	20.32	16.72	16.63	St.Dev
	10 ^b	51 ^c	25.4 ^d	7.75 ^e	Median
AA (N=31)	10.31	60.52	20.85	8.30	Mean
	11.90	20.75	15.53	13.98	St.Dev
	7.69 ^b	65 ^c	16.4 ^d	5.71 ^e	Median

^aSubsets are defined as naïve, Central Memory (CM), Effector memory (EM) and Effector Memory RA+ (EMRA)

^bP>0.05

^cP=0.0036 without adjusting for age and gender

^dP>0.05

^eP=0.0155 without adjusting for age and gender

Table 4

Public clonotypes for Caucasian Donors

Clonotype	ND001	ND006	ND008	ND018	ND019	ND020
CALW---DP---QELGKKIKVFGPGTKLIIT		2				1
CALW---GQ---ELGKKIKVFGPGTKLIIT		1			1	
CALW---V---QELGKKIKVFGPGTKLIIT		1	1			1
CALWE---A---ELGKKIKVFGPGTKLIIT	38	2	1			3
CALWE---AG---ELGKKIKVFGPGTKLIIT				1	1	
CALWE---AK---ELGKKIKVFGPGTKLIIT		6				2
CALWE---AP---QELGKKIKVFGPGTKLIIT					2	1
CALWE---A---QELGKKIKVFGPGTKLIIT	2	8	3	7	7	7
CALWE---AQ---LGKKIKVFGPGTKLIIT	1				1	
CALWE---AS---QELGKKIKVFGPGTKLIIT				1	1	
CALWE---D---QELGKKIKVFGPGTKLIIT			2	2	2	1
CALWE---DV---ELGKKIKVFGPGTKLIIT		1			1	
CALWE---E---ELGKKIKVFGPGTKLIIT	1		1	1		1
CALWE---EK---ELGKKIKVFGPGTKLIIT		1	2			
CALWE---ELGKKIKVFGPGTKLIIT			3			2
CALWE---E---QELGKKIKVFGPGTKLIIT		2				2
CALWE---G---QELGKKIKVFGPGTKLIIT		2	1	1		2
CALWE---I---QELGKKIKVFGPGTKLIIT			1			4
CALWE---K---ELGKKIKVFGPGTKLIIT					2	3
CALWE---L---QELGKKIKVFGPGTKLIIT		1	3		2	
CALWE---MQ---ELGKKIKVFGPGTKLIIT	1		9			
CALWE---NQ---ELGKKIKVFGPGTKLIIT		2		2		
CALWE---P---QELGKKIKVFGPGTKLIIT				3	1	2
CALWE---PQ---QELGKKIKVFGPGTKLIIT					5	1
CALWE---QELGKKIKVFGPGTKLIIT	1	4	6			
CALWE---RL---QELGKKIKVFGPGTKLIIT				1		1
CALWE---R---QELGKKIKVFGPGTKLIIT			1			1
CALWE---S---QELGKKIKVFGPGTKLIIT		2	1			1
CALWE---T---QELGKKIKVFGPGTKLIIT	4	3				2

Clonotype	ND001	ND006	ND008	ND018	ND019	ND020
CALWE---VD-----ELGKKIKVFGPGTKLIIT					23	1
CALWEV--E-----ELGKKIKVFGPGTKLIIT	1	2			6	2
CALWEV-----ELGKKIKVFGPGTKLIIT	5	4	2			2
CALWEV--G-----ELGKKIKVFGPGTKLIIT	1	4	1	2		1
CALWEV--H-----ELGKKIKVFGPGTKLIIT		5			1	
CALWEV--HQ-----LGKKIKVFGPGTKLIIT			1			1
CALWEV--K-----ELGKKIKVFGPGTKLIIT		1	1	1		1
CALWEV--LE-----ELGKKIKVFGPGTKLIIT			2		2	
CALWEV--LJ-----ELGKKIKVFGPGTKLIIT	9	1	4			
CALWEV--P-----ELGKKIKVFGPGTKLIIT	1		3	3		2
CALWEV--P-----QELGKKIKVFGPGTKLIIT	1			1		
CALWEV--QD-----ELGKKIKVFGPGTKLIIT		1		1		
CALWEV-----QELGKKIKVFGPGTKLIIT	5	20	25	13	7	19
CALWEV--QK-----LGKKIKVFGPGTKLIIT	1		1			1
CALWEV--Q-----QELGKKIKVFGPGTKLIIT		1		1		
CALWEV--R-----ELGKKIKVFGPGTKLIIT	2	4	25	2	1	5
CALWEV--RG-----ELGKKIKVFGPGTKLIIT		1	1			
CALWEV--RK-----LGKKIKVFGPGTKLIIT	3					1
CALWEV--R-----QELGKKIKVFGPGTKLIIT			1			2
CALWEV--SG-----LGKKIKVFGPGTKLIIT			1			1
CALWEV--T-----ELGKKIKVFGPGTKLIIT		1	2			1
CALWEV--WK-----LGKKIKVFGPGTKLIIT		1				1
Total # of public Jg1.2	77	84	105	43	66	79
Total # of Jg1.2	196	158	163	75	84	154
Fraction of public Jg1.2 (%)	39.29	53.16	64.42	57.33	78.57	51.30

Table 5

Public clonotypes for African-American Donors

Clonotypes	LR33	EG41	YL43	MP068	ND023	ND030	ND037	ND045
CAL WE---AK-----ELGKKIKVFGPGTKLIIT							1	1
CAL WE---AL-----QELGKKIKVFGPGTKLIIT							1	1
CAL WE---AP-----QELGKKIKVFGPGTKLIIT								1
CAL WE---A-----QELGKKIKVFGPGTKLIIT	3	2			5		1	5
CAL WE---AQ-----LGKKIKVFGPGTKLIIT		1	1					
CAL WE---AS-----QELGKKIKVFGPGTKLIIT	2				1			1
CAL WE---D-----QELGKKIKVFGPGTKLIIT					5			
CAL WE---E-----ELGKKIKVFGPGTKLIIT	2				3		1	
CAL WE---G-----QELGKKIKVFGPGTKLIIT	1	1	3	1			1	1
CAL WE---P-----QELGKKIKVFGPGTKLIIT		2			1	1		6
CAL WE-----QELGKKIKVFGPGTKLIIT		2						1
CAL WE---S-----QELGKKIKVFGPGTKLIIT					2			1
CAL WE---T-----QELGKKIKVFGPGTKLIIT	1		1					1
CAL WEV--A-----ELGKKIKVFGPGTKLIIT	1		6					3
CAL WEV--G-----ELGKKIKVFGPGTKLIIT		1			4		1	2
CAL WEV--GG-----LGKKIKVFGPGTKLIIT					1	30		
CAL WEV--H-----ELGKKIKVFGPGTKLIIT	1						61	2
CAL WEV--HG-----ELGKKIKVFGPGTKLIIT							5	2
CAL WEV--K-----ELGKKIKVFGPGTKLIIT					2			2
CAL WEV--LE-----ELGKKIKVFGPGTKLIIT		1					1	1
CAL WEV--L-----ELGKKIKVFGPGTKLIIT	2	1	1	1	1			2
CAL WEV--M-----ELGKKIKVFGPGTKLIIT			1					2
CAL WEV--N-----ELGKKIKVFGPGTKLIIT	1				2			
CAL WEV--P-----ELGKKIKVFGPGTKLIIT	2	2			2		1	
CAL WEV-----QELGKKIKVFGPGTKLIIT	5	6	9	11	18	73	4	15
CAL WEV--QK-----ELGKKIKVFGPGTKLIIT			1	1			1	
CAL WEV--Q-----QELGKKIKVFGPGTKLIIT		1	1					
CAL WEV--R-----ELGKKIKVFGPGTKLIIT	4	9	1		1		1	9
CAL WEV--RG-----ELGKKIKVFGPGTKLIIT					1			1

Clonotypes	LR33	EG41	YL43	MP068	ND023	ND030	ND037	ND045
Total # of public Jg1.2	25	21	32	14	48	109	80	59
Total # of Jg1.2	61	80	57	32	115	124	111	122
Fraction of public Jg1.2 (%)	41.0	26.3	56.1	43.8	41.7	87.9	72.1	48.4

Public Jγ1.2 public clonotypes among CA (Table 3) and AA (Table 4) donors

A total of 51 public Vγ2-Jγ1.2 clonotypes were found for CA donors (Table 3) and 29 public Vγ2-Jγ1.2 clonotypes were found for AA donors (Table 4). The number of occurrences in each donor is reported for every clonotype and the fraction of Jγ1.2 sequences coding public clonotypes is calculated as: total number of Jγ1.2 sequences % total number of Jγ1.2 sequences coding public clonotypes. Highlighted clonotypes are present as public in both groups of donors. The clonotype in bold represents a germline encoded sequence, and it is present in all donors.